Award Number: W81XWH-10-1-0023

TITLE:
"Does RBC Storage Age Effect Inflammation, Immune Function and Susceptibility to Transfusion Associated Microchimerism in Critically Ill Patients? Adverse Effects of RBC Storage in Critically Ill Patients"

PRINCIPAL INVESTIGATOR: Philip C. Spinella, MD

CONTRACTING ORGANIZATION: BLOOD SYSTEMS, INC.
SCOTTSDALE AZ 85257-1101

REPORT DATE: December 2014

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	2. REPORT TYPE	3. DATES COVERED *(From - To)*
December 2014	Annual	09 Nov 13 - 08 Nov 2014

4. TITLE AND SUBTITLE
"Does RBC Storage Age Effect Inflammation, Immune Function and Susceptibility to Transfusion Associated Microchimerism in Critically Ill Patients?
Adverse Effects of RBC Storage in Critically Ill Patients"

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-10-1-0023

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Philip Spinella, MD

Philip J. Norris, MD

email: spinella_p@kids.wustl.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
BLOOD SYSTEMS, INC.
6210 E OAK ST
SCOTTSDALE, AZ
85257-1101

8. PERFORMING ORGANIZATION REPORT NUMBER 4

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research
And Materiel Command
Fort Detrick, Maryland
21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for public release; distribution unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

The study aim is to investigate specific mechanisms of potential adverse effects related to RBC storage age in critically ill patients. Enrollment has been completed since the last report. As of February 4, 2015, we have enrolled 100 of our goal of 100 subjects for testing of immune and coagulation parameters, and 153 of a goal of 200 patients for microchimerism testing. Samples have been collected, processed, and shipped from Canada to the repository at Blood Systems Research Institute (BSRI) in San Francisco, California. The repository consists of plasma, PBMCs and whole blood samples. Cytokine and coagulation testing has been completed for all 100 subjects outlined in the statement of work. Analysis of coagulation, micro particle, microchimerism and immune function testing on study samples is underway, and will be correlated with clinical outcome data shortly when that becomes available.

15. SUBJECT TERMS
RBC storage age; subject enrollment; IRB approval; repository; Microparticles; Coagulation; Microchimerism

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
U					USAMRMC
a. REPORT	b. ABSTRACT	c. THIS PAGE	UU	11	19b. TELEPHONE NUMBER *(include area code)*
U	U	U			

Table of Contents

Adverse Effects of RBC Storage in Critically Ill Patients

INTRODUCTION

Critically ill patients are specifically at risk of adverse effects resulting from the use of RBCs of increased storage age. A large multicenter randomized controlled trial in 30 Canadian centers of 2500 critically ill patients called the Age of Blood Evaluation (ABLE) trial has been completed. In this trial of critically ill patients, which included patients with traumatic injuries, study groups were randomized to either RBCs of < 8 days storage time or standard RBC storage time. The primary outcome of this trial is 90 day mortality. Secondary outcomes include severity of multiple organ dysfunction syndrome, serious thrombotic events and nosocomial infections, and ICU and hospital length of stay. Prospective clinical studies investigating the mechanisms and clinical outcomes associated with increased or decreased RBC storage age in critically ill patients including traumatic injury have not been performed. The ABLE study presents a unique and probably one-time opportunity to investigate mechanisms in the context of clinical outcomes for well-characterized study groups. Our ancillary study is designed to determine specific mechanisms of adverse effects related to the RBC storage age in transfused critically ill patients enrolled in the ABLE study. Specifically we will determine if the RBC unit storage time affects patient's immune function, inflammation, coagulation, microparticle concentrations and microchimerism.

Since the last annual report, preliminary data from the ABLE trial have been reported in abstract form. The primary endpoint of the trial showed no difference in 90-day mortality between the fresh and standard issue RBC units. Our site is still blinded to clinical outcome of the participants, but we expect to be unblinded shortly so that formal statistical analyses can begin. Additionally, we will receive data on clinical outcomes of trial participants, which will allow us to pursue our secondary hypotheses outlined in Aim 1d below.

Hypotheses

1.) Increased storage time of transfused RBC units will affect both inflammation and coagulation factors in critically ill patients and these parameters will be positively associated with measured clinical endpoints including increased morbidity (sepsis, serious thrombotic events, multi-organ failure) and mortality.

Aims

1.) To determine how RBC unit storage time affects inflammation and coagulation in critically ill patients, how these effects change over time after transfusion and if these parameters correlate with clinical outcomes.

1a.) To determine how RBC unit storage time affects immune function in critically ill patients, and how these effects change over time and if changes in immune function correlate with clinical outcomes.

1b.) To determine if RBC unit storage time affects microparticle concentrations in the critically ill and if microparticle concentrations correlate with increased inflammation, coagulation, altered immune function and clinical outcomes.

1c.) To measure an extensive profile of coagulation markers to determine if increased inflammation leads temporally to hypercoagulation and increased risk of multi-organ failure and death.

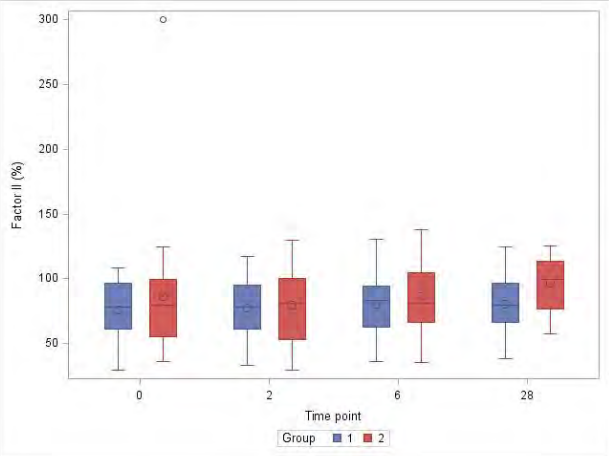
1d.) To determine the incidence and magnitude of transfusion associated microchimerism

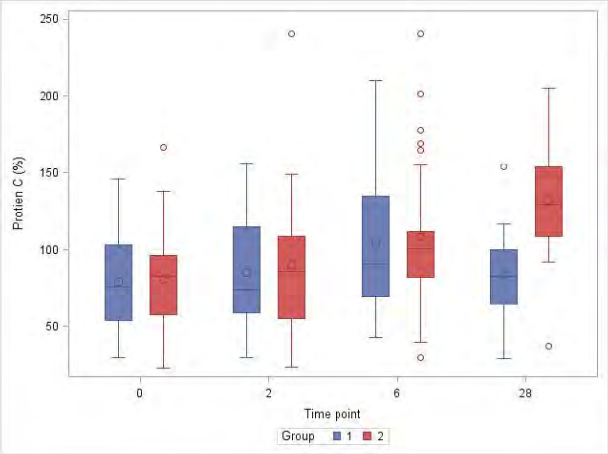
2.) To develop a patient sample repository for future analysis of additional effects of RBC storage age in critically ill patients.

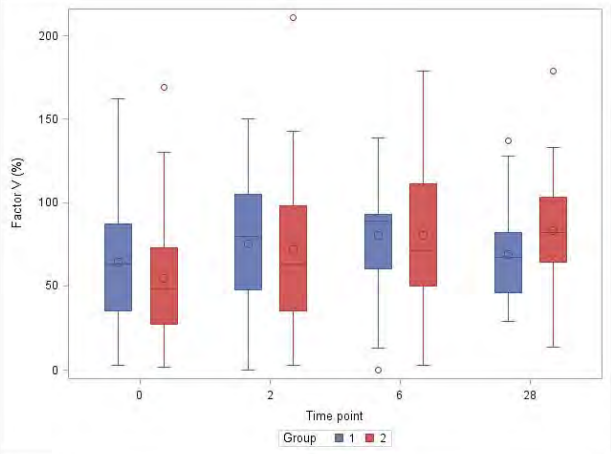
BODY

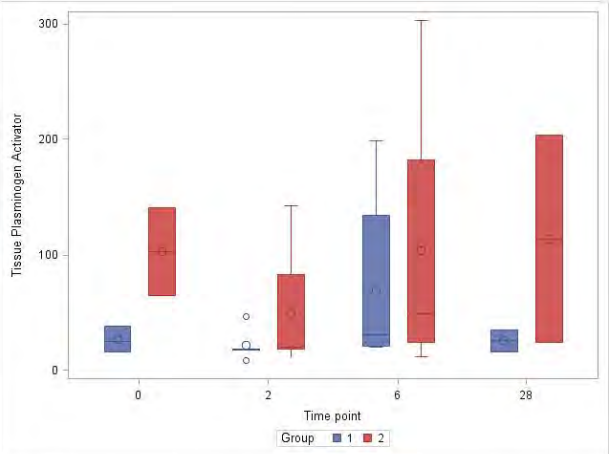
Since the last annual report, we have continued to enroll patients and collect samples at all sites. Enrollment has been completed since the last report. As of February 4, 2015, we have enrolled 100 of our goal of 100 subjects for testing of immune and coagulation parameters, and 153 of a goal of 200 patients for microchimerism testing. As will be discussed below, all microchimerism testing to date has been negative, so the shortfall of 47 subjects for microchimerism testing will not have a significant effect on the conclusions of the study.

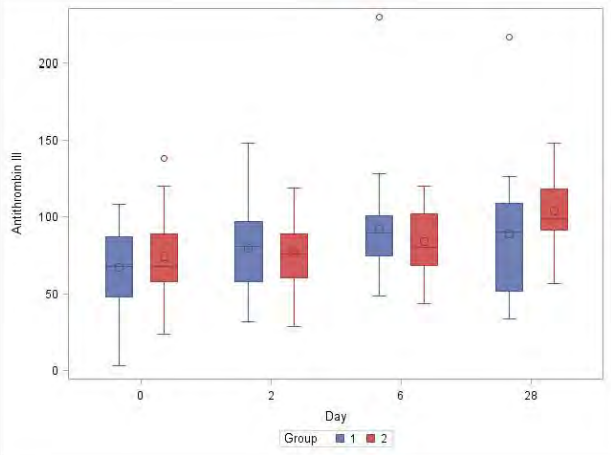
Evaluable samples have been collected, processed and shipped from the clinical sites to BSRI. These samples are being stored at BSRI. Cytokine and coagulation testing has been completed for all 100 subjects outlined in the statement of work. We have performed preliminary analysis of the coagulation testing for 65 of patients (see Fig. 2 for representative markers). The results are categorized as Group 1 and 2, since we are still blinded to the treatment groups of the parent RCT. This information along with correlation with clinical outcomes will be available shortly since the study is completed. For most parameters in the samples tested, there seems to be a similarity in change over time between the two study groups. Further analysis on significance of the differences seen here is currently underway.

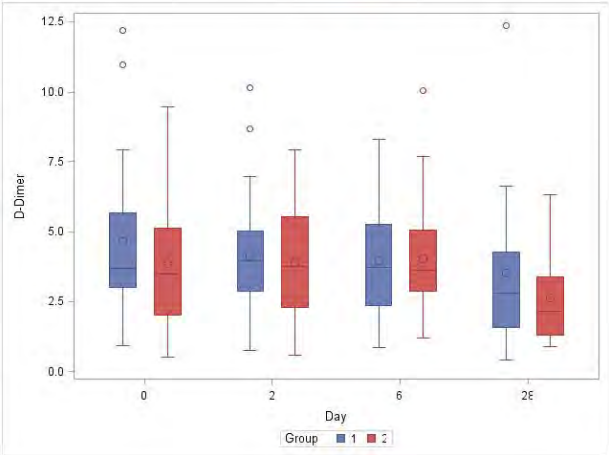

Factor II (%)
300
250
200
150
100
50
0
2
6
28
Time point
Group 1 2

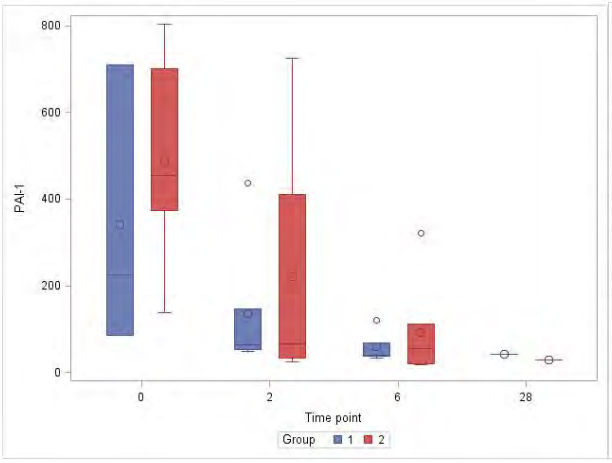

Protien C (%)
250
200
150
100
50
0
2
6
28
Time point
Group 1 2

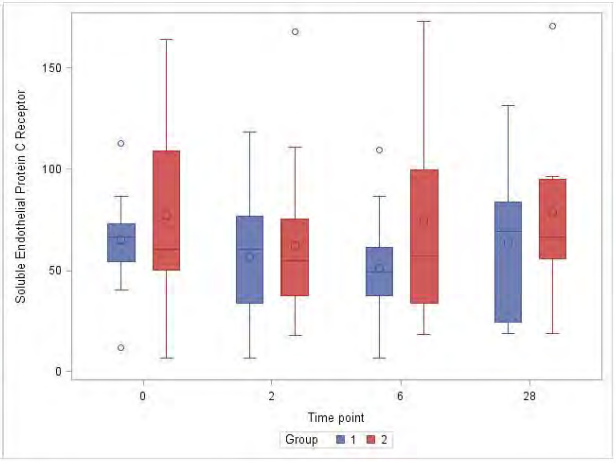

Factor V (%)
200
150
100
50
0
0
2
6
28
Time point
Group 1 2

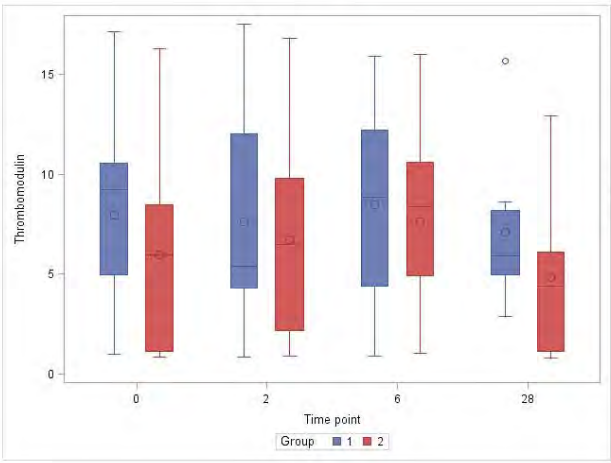

Tissue Plasminogen Activator
300
200
100
0
0
2
6
28
Time point
Group 1 2

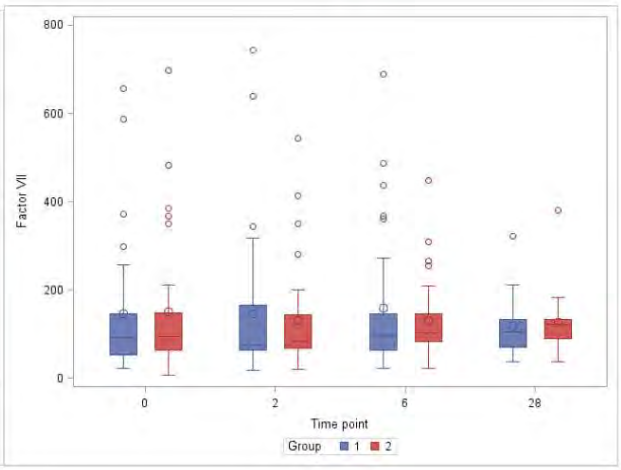

Antithrombin III
200
150
100
50
0
0
2
6
28
Day
Group 1 2

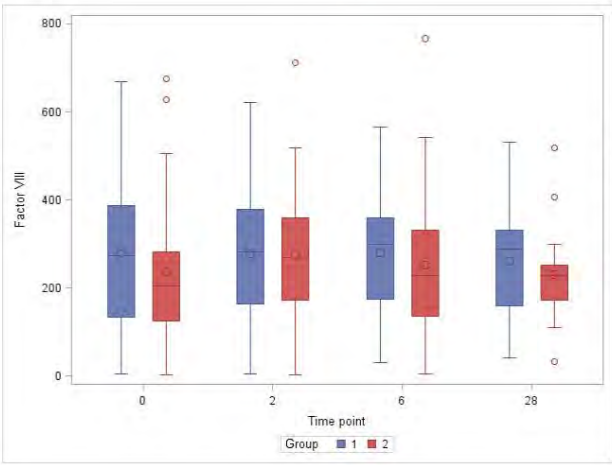

D-Dimer
12.5
10.0
7.5
5.0
2.5
0.0
0
2
6
28
Day
Group 1 2

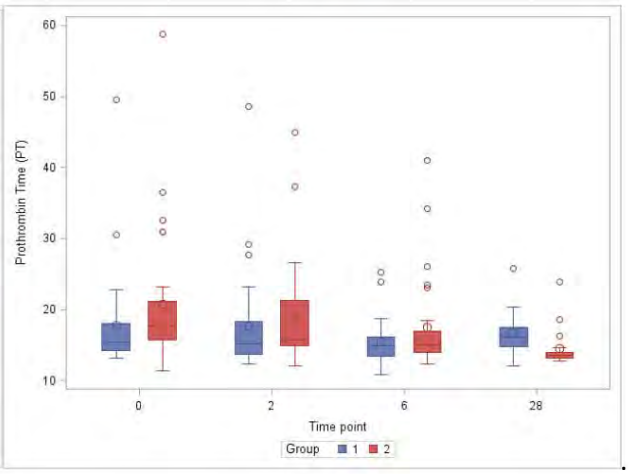

PAI-1
800
600
400
200
0
0
2
6
28
Time point
Group 1 2


Soluble Endothelial Protein C Receptor
150
100
50
0
0
2
6
28
Time point
Group 1 2


Thrombomodulin
15
10
5
0
0
2
6
28
Time point
Group 1 2


Factor VII
800
600
400
200
0
0
2
6
28
Time point
Group 1 2


Factor VIII
800
600
400
200
0
0
2
6
28
Time point
Group 1 2


Prothrombin Time (PT)
60
50
40
30
20
10
0
2
6
28
Time point
Group 1 2

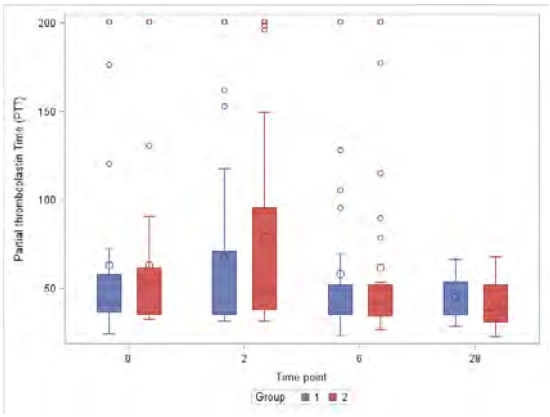


Fig.2 Coagulation parameters tested for 65 subjects for Day 0, 2, 6 and 28. Patients either received fresh blood or standard unit (which group is which is unknown).

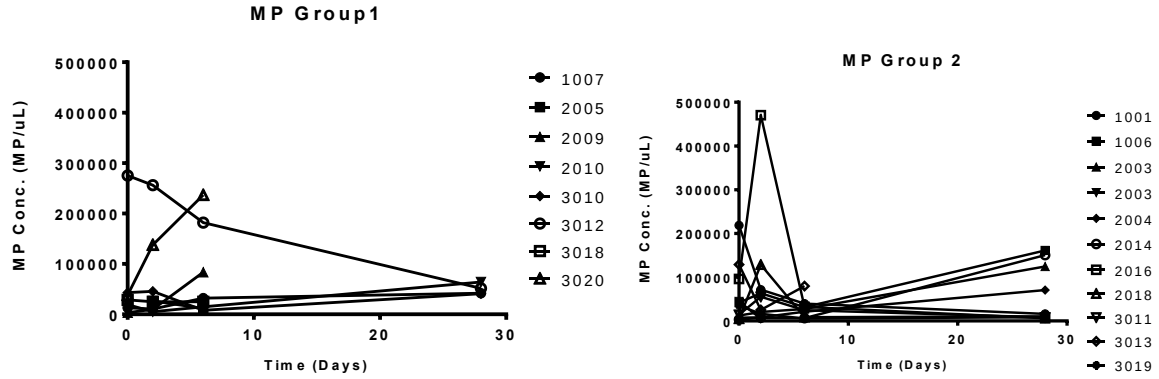


Fig. 3 Microparticle counts of the first 19 subjects tested. Patients in Groups 1 and 2 received either fresh blood (< 8 days storage time) or standard issue/oldest in inventory. Samples were tested at Days 0, 2, 6, and 28. Control MPs were tested using MPs pooled from 3 normal donors. Preliminary results show early elevated MPs in some subjects, with late elevations in others compared to controls. Changes appear to be more pronounced in Group 2 than Group 1.

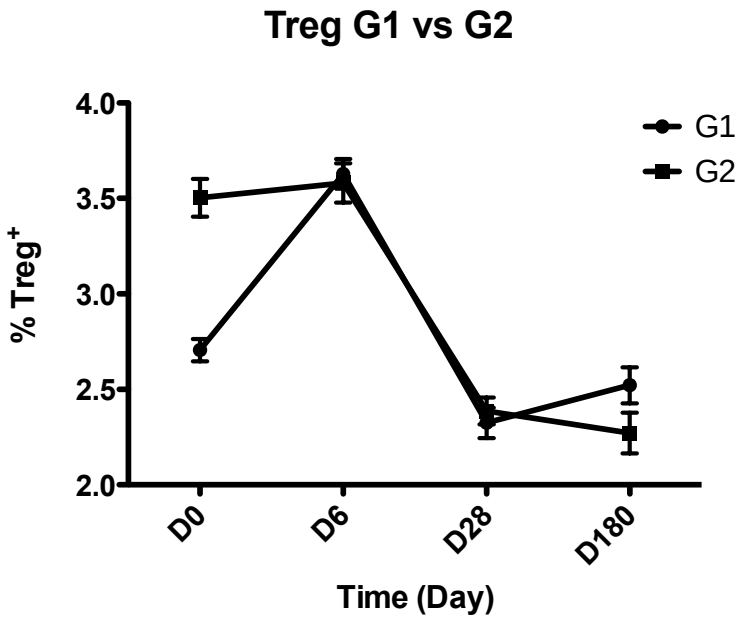
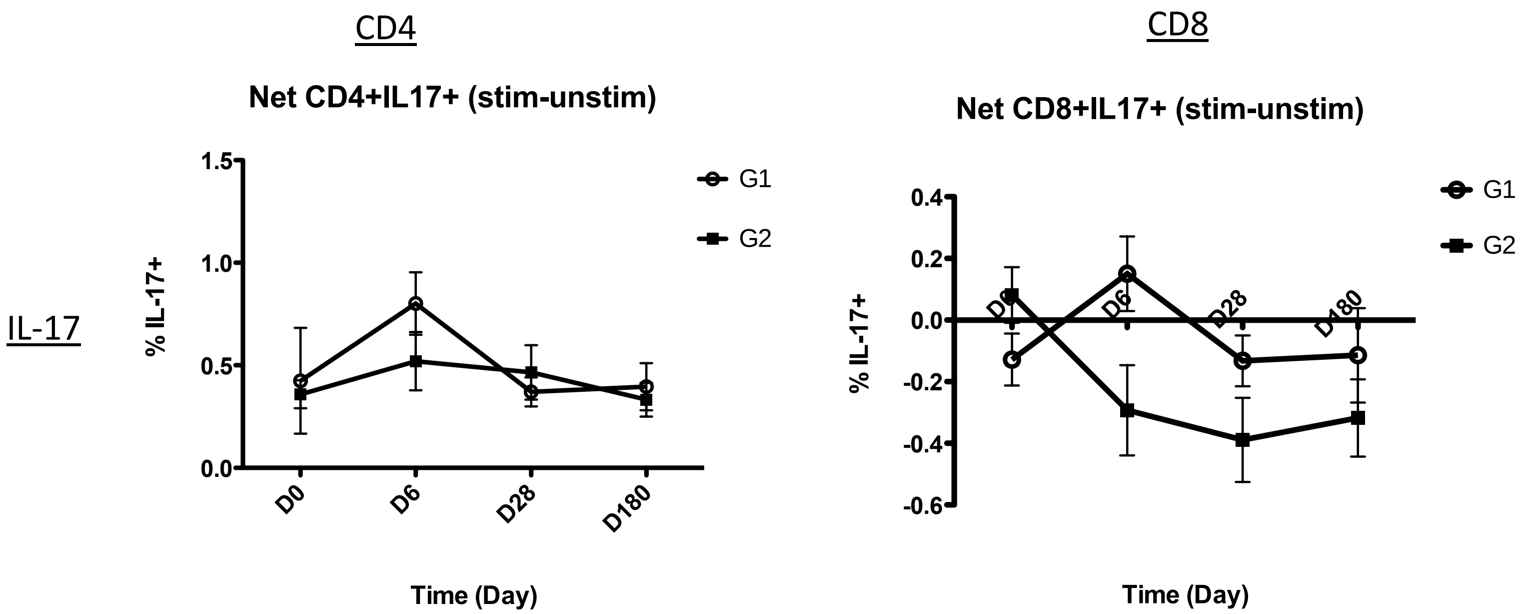
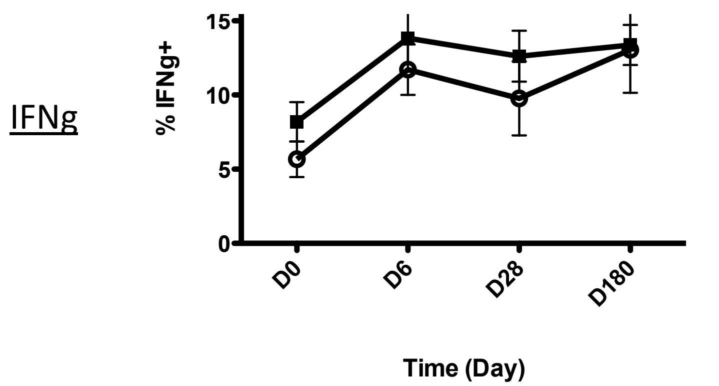
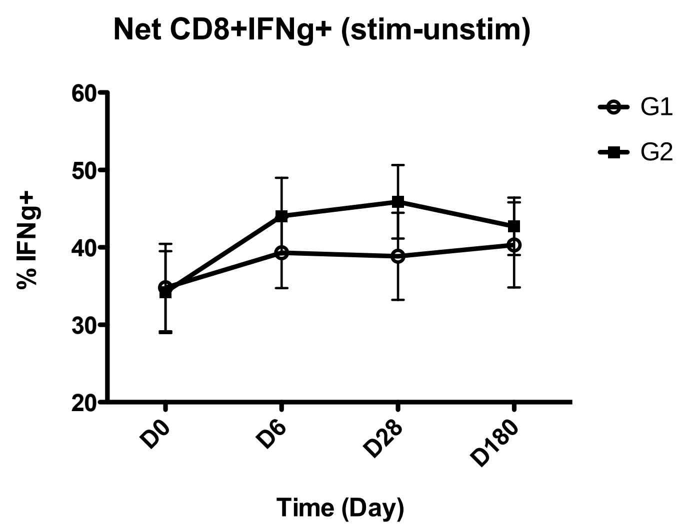


Fig. 4 Evolution of Treg markers in 99 patients. Patients in Groups 1 and 2 received either fresh blood **(**< 8 days storage time) or standard issue/oldest in inventory. Samples were tested at Days 0, 6, 28, and 180. Results demonstrate reduction in Treg markers in both groups over time.



	G1			G2		
	Mean	SEM	N	Mean	SEM	N
D0	0.424	0.258	18	0.359	0.068	23
D6	0.802	0.152	28	0.520	0.142	25
D28	0.371	0.071	15	0.466	0.133	23
D180	0.396	0.115	14	0.333	0.083	16





G1 G2 G1

G2	
SEM	N
5.282	22
4.940	27
4.752	23
3.707	15

Fig. 5 Evolution of Th17 markers in 91 patients. Patients in Groups 1 and 2 received either fresh blood (< 8 days storage time) or standard blood (mean 21 days storage time). Samples were tested at Days 0, 6, 28, and 180. Please note that these do not include results for all samples tested due to reasons stated above.

From the first 100 patients enrolled, 59 patients had day 28 samples collected and 31 had day 180 samples collected (informative samples from 65 total patients). In addition to these samples (whose testing is summarized below), 48 samples from the second cohort of 53 enrolled subjects contained informative day 28 and/or 180 samples. Testing for microchimerism is underway for these samples. The HLA-DR and InDel types of each subject were determined using (pre-transfusion) Day 0 samples in order

to identify the subjects' type prior to transfusion and to identify which alleles or polymorphisms will be informative. The follow-up post-transfusion samples were then probed for presence of microchimeric DNA or donor derived DNA using informative alleles or polymorphisms. Typing using the HLA-DR and InDel panels is completed for all Day 0 samples. No microchimerism was detected in all Day 28 and 180 samples when amplified for InDel polymorphisms. We are currently in the process of determining whether microchimeric DNA is present in the last 8 subjects using HLA-DR informative alleles.

KEY RESEARCH ACCOMPLISHMENTS

- At the end of enrollment, 100 of 100 patients for immunological and coagulation studies were enrolled.
- Fifty-three of a planned 100 additional subjects for microchimerism testing were enrolled.
- Testing for all immunology and coagulation studies has been completed.
- We have completed microparticle testing on all samples from 65 patients.
- We are in the process of completing microchimerism testing for 37 patients.

REPORTABLE OUTCOMES

We have continued building a repository of plasma, PBMCs and whole blood samples. Analysis of coagulation, micro particle, microchimerism and immune function testing on study samples is underway, and will be correlated with clinical outcome data shortly when that becomes available. In addition, two manuscripts detailing how microparticles will be measured in this study have been accepted for publication (see References).

CONCLUSION

The ABLE ancillary study has finished enrollment of patients and collection of samples. There is a small amount of microparticle testing to be completed, otherwise laboratory testing is complete. Once clinical data becomes available to link to the laboratory data, formal statistical analysis will begin.

REFERENCES

1. Inglis HC, Danesh A, Shah A, Lacroix J, Spinella PC, Norris PJ. Techniques to improve detection and analysis of extracellular vesicles using flow cytometry. **Cytometry A** (in press).
2. Inglis HC, Norris PJ, Danesh A. Techniques for the analysis of extracellular vesicles using flow cytometry. **J Vis Exp** (in press).

APPENDICES

None